development of effective approaches to treatment is a primary concern in the field of medicine.

Summary of the Invention

The invention provides methods of treating diabetes (type 1 diabetes or type 2 diabetes) in patients, which involve administering to the patients a hydroxylated amino acid (for example, 4-hydroxyisoleucine, e.g., the 2S,3R,4S isomer of 4-hydroxyisoleucine) and one or more additional antidiabetic agents, to obtain an improved (e.g., synergistic or additive) effect. Examples of additional antidiabetic agents that can be used in the invention include biguanides (e.g., metformin), sulfonylurea drugs, glinides, glitazones (e.g., thiazolidinediones, such as rosiglitazone maleate), glucagon-like peptide 1 receptor agonists (e.g., Exenatide®), and insulin. Other examples of antidiabetic (and other) agents that can be used in combination with hydroxylated amino acids according to the invention are listed below. In one example, 4-hydroxyisoleucine is combined with insulin and/or metformin, while in another example, 4-hydroxyisoleucine is combined with metformin and/or a thiazolidinedione. The hydroxylated amino acid and other antidiabetic agents can be administered at or about the same time as one another or at different times. Also included in the invention are pharmaceutical kits and compositions (e.g., tablets or capsules) that include combinations of the agents noted above and elsewhere herein.

The invention provides a method of treating diabetes in a patient, the method comprising administering to the patient 4-hydroxyisoleucine and one or more additional antidiabetic agents selected from the following types of antidiabetic agents: biguanides, sulfonylurea drugs, glinides, insulin-sensitizing agents, glucagon-like peptide 1 receptor agonists, agents that slow carbohydrate absorption, glucagon antagonists, glucokinase activators, imidazolines, glycogen phosphorylase inhibitors, oxadiazolidinediones, dipeptidyl peptidase-IV inhibitors, protein tyrosine phosphatase inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis or glycogenolysis, glucose uptake modulators, glycogen synthase kinase-3 inhibitors, antihyperlipidemic agents, antilipidemic agents, peroxisome proliferator-activated receptor agonists, retinoid X receptor agonists, and antihypertensive agents.

The invention provides use of 4-hydroxyisoleucine and one or more antidiabetic agents in the manufacture of a medicament for treating diabetes, wherein the additional antidiabetic agent(s) is selected from the following types of antidiabetic agents:

biguanides, sulfonylurea drugs, glinides, insulin-sensitizing agents, glucagon-like peptide 1 receptor agonists, agents that slow carbohydrate absorption, glucagon antagonists, glucokinase activators, imidazolines, glycogen phosphorylase inhibitors, oxadiazolidinediones, dipeptidyl peptidase-IV inhibitors, protein tyrosine phosphatase inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis or glycogenolysis, glucose uptake modulators, glycogen synthase kinase-3 inhibitors, antihyperlipidemic agents, antilipidemic agents, peroxisome proliferator-activated receptor agonists, retinoid X receptor agonists, and antihypertensive agents.

The invention provides a pharmaceutical kit comprising 4-hydroxyisoleucine and one or more antidiabetic agents selected from the following types of antidiabetic agents: biguanides, sulfonylurea drugs, glinides, insulin-sensitizing agents, glucagon-like peptide 1 receptor agonists, agents that slow carbohydrate absorption, glucagon antagonists, glucokinase activators, imidazolines, glycogen phosphorylase inhibitors, oxadiazolidinediones, dipeptidyl peptidase-IV inhibitors, protein tyrosine phosphatase inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis or glycogenolysis, glucose uptake modulators, glycogen synthase kinase-3 inhibitors, antihyperlipidemic agents, antilipidemic agents, peroxisome proliferator-activated receptor agonists, retinoid X receptor agonists, and antihypertensive agents.

The invention provides a pharmaceutical composition comprising 4-hydroxyisoleucine, one or more antidiabetic agents and a pharmaceutically acceptable excipient, wherein said additional antidiabetic agent(s) is selected from the following types of antidiabetic agents: biguanides, sulfonylurea drugs, glinides, insulin-sensitizing agents, glucagon-like peptide 1 receptor agonists, agents that slow carbohydrate absorption, glucagon antagonists, glucokinase activators, imidazolines, glycogen phosphorylase inhibitors, oxadiazolidinediones, dipeptidyl peptidase-IV inhibitors, protein tyrosine phosphatase inhibitors, inhibitors of hepatic enzymes involved in stimulation of gluconeogenesis or glycogenolysis, glucose uptake modulators, glycogen synthase kinase-3 inhibitors, antihyperlipidemic agents, antilipidemic agents, peroxisome proliferator-activated receptor agonists, retinoid X receptor agonists, and antihypertensive agents.

The invention provides the use of a pharmaceutical kit of this invention or of a pharmaceutical composition of this invention, for treating diabetes in a patient.

The invention provides several advantages. For example, because the drug combinations described herein are used to obtain improved (e.g., synergistic or additive)

effects, it is possible to consider administering less of each drug, leading to a decrease in the overall exposure of patients to drugs, as well as any untoward side effects of any of the drugs. In addition, greater control of the disease may be achieved, because the drugs can combat the disease through different mechanisms.

Other features and advantages of the invention will be apparent from the following detailed description and the claims.

Brief Description of the Drawings

Figure 1 is a bar graph showing additive stimulation of glucose uptake in 3T3-L1 differentiated adipocytes by the combination of insulin and ID 1101. Cells were exposed to the treatments for 0.5h, 1h, 2h, 4h or 5h. Treatments were: (1) Control; (2) 0.5 mM ID 1101; (3) 1 mM ID 1101; (4) Insulin 10^{-7} M; (5) 0.5 mM ID 1101 + Insulin 10^{-7} M; (6) 1 mM ID 1101 + Insulin 10^{-7} M.

Figures 2A, 2B, 2C and 2D are bar graphs showing changes in plasma glucose levels from baseline during an oral glucose tolerance test. AUC Delta OGTT is shown at Day 0 (Fig.2A), at Day 7 (Fig.2B); at Day 14 (Fig.2C) and at Day 21 (Fig. 2D). Treatments were: (1) Control NDC; (2) ID 1101 50 mg/kg BID; (3) ID 1101 100 mg/kg BID; (4) Rosiglitazone 1.5 mg/kg BID; (5) Rosiglitazone 5 mg/kg BID; (6) ID 1101 50 mg/kg + Rosiglitazone 1.5 mg/kg BID; (7) Control DIO.

Figure 3 is a bar graph showing the effect on insulin secretion in INS-1 beta cells of ID 1101 in combination with Glibenclamide. Treatments were: (1) 4.5 mM Glucose; (2) 0.1 mM ID 1101; (3) Glibenclamide 10^{-11} M; (4) 0.1 mM ID 1101 + Glibenclamide 10^{-11} M; (5) Glibenclamide 10^{-10} M; (4) 0.1 mM ID 1101 + Glibenclamide 10^{-10} M.

Figure 4 is a bar graph showing the effect on insulin secretion in INS-1 beta cells of ID 1101 in combination with 10^{-10} M or 10^{-9} M Exendin-4. White bars: Control buffer; Dashed bars: 0.01 mM ID 1101; Black bars: 0.5 mM ID 1101.

Detailed Description of the Invention

The invention provides methods and pharmaceutical kits or compositions for use in treating diabetes and related diseases or conditions, such as metabolic syndrome. The invention is based on the administration of hydroxylated amino acids, such as 4-hydroxyisoleucine, to patients with one or more other antidiabetic agents, in order to obtain an improved (e.g., synergistic or additive) effect. As is discussed further below, examples of agents that can be administered with hydroxylated amino acids, such as 4-hydroxyisoleucine, according to the invention, include insulin, biguanides, sulfonylureas, glinides, glitazones, glucagon like peptide-1 (GLP-1) and agonists thereof, agents that slow carbohydrate absorption, glucagon antagonists, glucokinase activators, and other agents mentioned herein. The methods and compositions of the invention are described in further detail, as follows.

Hydroxylated Amino Acids

Central to the invention is the administration of one or more hydroxylated amino acids (e.g., mono-hydroxylated amino acids, poly-hydroxylated amino acids, or lactonic forms of such hydroxylated amino acids), in combination with one or more other antidiabetic agents, to patients. A specific example of a hydroxylated amino acid that can be used in the invention is 4-hydroxyisoleucine (e.g., the 2S,3R,4S isomer), which has been shown both to stimulate insulin secretion in a glucose dependent manner, and to decrease insulin resistance (see, e.g., U.S. Patent No. 5,470,879; WO 01/15689; Broca et al., Am. J. Physiol. 277:E617-E623, 1999; the teachings of each of which are incorporated herein by reference).

4-hydroxyisoleucine for use in the invention can be obtained, for example, by chemical synthetic methods. However, this compound is naturally present in high quantities in the seeds of the legume fenugreek (*Trigonella foenum-graecum L.*), from which it can be purified using methods such as those described in U.S. Patent No. 5,470,879, WO 97/32577, WO 01/72688, and Wang et al., Eur. J. Org. Chem. 834-839, 2002, the teachings

Objective:

The objective of this study was to determine the effect of Rosiglitazone and ID 1101, alone and in combination, on glucose tolerance in mice rendered hyperglycemic by consuming a high fat diet.

Materials and Methods:

C57BL6 mice were received at 7-8 weeks of age and fed a high fat diet (45% of calories from fat) for 8 weeks. Blood glucose was checked and animals with readings between 200 and 220 mg/dL were randomized into control and treatment groups. A group of C57BL6 mice receiving a normal diet was included as a control.
Treatment groups included those receiving twice daily treatment by oral gavage with Rosiglitazone (1.5 or 5 mg/kg), ID 1101 (50 or 100 mg/kg), or a combination of Rosiglitazone and ID 1101 (1.5 and 50 mg/kg, respectively).

A baseline oral glucose tolerance test (OGTT) was administered prior to commencement of treatment. The test was repeated on days 7, 14, and 21, to determine whether the treatments influenced glucose tolerance.

Results:

As expected, the baseline OGTT showed that the animals receiving the high fat diet exhibited less tolerance to the glucose challenge than did the normal diet control (NDC) animals ($p<0.05$) (Figures 2A, 2B, 2C and 2D). On day 7, the animals underwent an OGTT and the results were compared between groups. The animals treated with the combination of ID 1101 (50 mg/kg) and Rosiglitazone (1.5 mg/kg) were significantly more tolerant to the glucose challenge relative to the high fat diet control animals (DIO) ($p<0.05$). Similarly, animals treated with Rosiglitazone at 5 mg/kg also were more glucose tolerant that the high fat diet control animals ($p<0.05$). While there was a trend indicating the drug combination may be more efficacious, the outcome was not statistically significant.

Results of the Day 14 OGTT showed a similar but non-significant trend. However, by Day 21, only the mice receiving Rosiglitazone (1.5 or 5 mg/kg) showed significantly improved glucose tolerance relative to the high fat diet control animals ($p<0.05$)